Evaluating the genetic relationship between spondyloarthritis and human plasma proteins: Linkage disequilibrium score regression

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Abstract

Background: Spondyloarthritis (SpA) is a kind of complex bone disease influenced by genetic factors, environment, and lifestyles. In recent years, the impact of human plasma proteins on the development of SpA has been reported. However, the genetic relationships between human plasma proteins and SpA have not systematically studied.

Methods: The genome-wide association study (GWAS) summary dataset of SpA was derived from a published study, involving a total of 452,264 White British individuals (3,966 SpA patients and 448,298 controls). The GWAS summary dataset for 3,622 human plasma proteins was derived from the published study. Consequently, linkage disequilibrium score regression (LD score regression) analysis was performed to evaluate the genetic correlations between each human plasma protein and SpA. The significant plasma proteins were further compared with the differentially expressed genes (DEGs) detected by the mRNA expression profiles of SpA.

Results: For LD score regression analysis, 5 kinds of human plasma proteins were found to be associated with SpA, such as Testis-specific chromodomain protein Y 1(r_g =0.2973, *P* value=0.0439), Interleukin-17D (r_g =0.3890, *P* value=0.0381), Neurensin-1(r_g =0.3667, *P* value=0.0333), Trypsin-2(r_g =0.3653, *P* value=0.0380), Serpin A9(r_g =0.3746, *P* value=0.0424). Among them, IL-17D is the common gene identified for SpA both at protein level and gene expression level ($P_{protein}$ value=0.0006, P_{mRNA} value=0.0381).

Conclusions: We identified several SpA associated human plasma proteins. Our novel findings may provide new ideas for the future study of the genetic mechanism of SpA.

Introduction

The term spondyloarthritis (SpA) encompasses a group of chronic inflammatory diseases that exhibit common features in terms of clinical presentation and genetic predisposition[1]. The pathological characteristics of SpA are the inflammation at the enthesis, synovium, and the adjacent bone[2]. The prevalence of SpA and show considerable differences among ethnic groups and populations, which varied from 0.01% in Japan to 2.5% in Alaska[3]. The annual estimated incidence of SpA was 62.5/100.000[3].

The SpA is a kind of complex bone disease influenced by genetic factors, environment, and lifestyles. The Genetic factor plays an essential role in SpA. Previous studies have estimated that genetic risk factors contribute to 80–90% of the susceptibility to ankylosing spondylitis[4]. In the last few years, our understanding of genetic susceptibility to SpA has dramatically improved thanks to the findings derived from powered genome-wide association studies (GWASs) based on single nucleotide polymorphism (SNP) arrays[1]. However, the genetic mechanism of SpA is complicated and remains primarily unknown now.

The human plasma proteins are a group of proteins in plasma. So far, more than 3600 plasma proteins have key roles in various biological processes, including signaling, transport, growth, repair, and defense against infection[5]. The human plasma proteins play a significant role as signals or biological markers in disease diagnosis and therapeutic monitoring[6]. In recent years, the impact of human plasma proteins on the development of SpA has been reported. For example, a strong genetic association between SpA and human leukocyte antigen-B27 (HLA-B27) has been shown[7]. Grandon B et al. showed the antagonistic interaction of HLA-B27 with ALK2, which exerts inhibitory functions on the TGFβ/BMP signaling pathway at the cross-road between inflammation and ossification, could adequately explain the pathological mechanisms of SpA[7]. Lymphotoxin-a (LTA) is a proinflammatory cytokine that can cause tissue injury. Recently, researchers found that LTA may be associated with the pathogenesis of ankylosing spondylitis, which provides new clues for understanding the pathogenesis mechanism of ankylosing spondylitis[8]. But until now, there were few studies on the relationship between human plasma proteins and SpA.

Linkage disequilibrium score regression (LD score regression) analysis is one of the bioinformatics methods to identify the genetic correlation among the multiple traits and distinguish between bloated test statistics from confounding bias and polygenicity in GWAS[9]. The more important use of LD score regression analysis is the estimation and correction of confusion[9]. In recent years, researchers have made significant progress in studying the genetic correlation between complex diseases and traits by using LD score regression analysis. For example, Winiger EA et al. identified some genes both associated with cannabis use and sleep deficits and proved the cannabis use is genetically associated with sleep deficits by using LD score regression analysis[10]. In addition, Liu L et al. evaluated the genetic correlations between human plasma proteins and different sites of OA from the GWAS summary datasets of 3,622 plasma proteins and 44640 hospital-diagnosed OA patients through using LD score regression analysis[11].

In this study, we used the large-scale GWAS summary datasets of SpA and human plasma proteins to find associated human plasma proteins by analyzing the genetic correlation among them. Then GEO2R tool was performed to identify differentially expressed genes(DEGs) which differently expressed between SpA patients and controls. Our study provide new insights into the genetic mechanism, diagnosis, and treatment of SpA.

Material And Methods

The GWAS summary dataset of SpA

The large-scale GWAS summary dataset of SpA was obtained from the previously published study[12]. In brief, 4,033 diagnosed SpA and 458,900 controls of European analyzed in this study. The UK Biobank participants genotyped using the Affymetrix UK BiLEVE Axiom or UK Biobank Axiom array[12]. The data augmented by the imputation of ~ 90 million genetic variants from the Haplotype Reference Consortium, the 1,000 Genomes Project, and the UK 10K project [13, 14]. After quality control, the final study cohort

included 452,264 samples, including 3,966 SpA and 448,298 controls. Detailed information about the genotyping, imputation, and quality control can be found in the published study[12].

The GWAS summary dataset of human plasma proteins

The GWAS summary dataset of human plasma proteins was obtained from the previously published study[5]. In brief, 3,622 plasma proteins were quantified in 3,301 healthy participants from 25 centers across England[5]. Genotyping was performed on the Affymetrix Axiom UK Biobank genotyping array. Imputation was performed via the Sanger Imputation Server by using a combined 1,000 Genomes Phase3-UK10K reference panel[5]. Simple linear regression using an additive genetic model to test genetic associations. After quality control, the GWAS summary dataset of 3,283 plasma proteins used in the following genetic correlation analysis[5]. The detailed information about samples, imputation can be found in the published study[5].

The genetic correlations between SpA and human plasma proteins

We used the LD score regression software (https://github.com/bulik/ldsc) to evaluate the genetic correlations between SpA and human plasma proteins. LD score regression is a method to estimate the genetic correlation by using GWAS summary statistics rather than individual-level genotype data[9, 15]. LD score regression can distinguish between true polygenes and mixed biases[15]. If the genetic association is statistically and quantitatively significant, we can be sure that the overall phenotypic association is not entirely attribute to the environmental confounding factors[15]. After correcting for multiple testing, the significant threshold of this study should set at P = 0.05.

Screening of DEGs related to SpA

The Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/GEO/) is the largest fully public repository for high-throughput molecular abundance data, primarily gene expression data[16, 17]. GEO2R tool(https://www.ncbi.nlm.nih.gov/geo/geo2r/) is an R-based web application that helps users analyze the GEO data. In this study, we used the GEO2R tool to identify differentially expressed genes (DEGs) which were differently expressed between SpA patients and controls. We found a dataset named GSE57376, which contains 25 samples, including 15 patients with psoriasis who did not have psoriatic arthritis and 10 patients with psoriatic arthritis[18]. DEGs were identified when the following two conditions were met: adjusted P value of < 0.05 by the moderated t statistic and $|\log2FC| > 1$. We identified DEGs to verify whether the human plasma proteins have genetic correlations with SpA at the gene expression level.

Results

The genetic correlations between SpA and human plasma proteins

We detected 5 human plasma proteins have significant genetic correlations with SpA (Table 1), they are Testis-specific chromodomain protein Y 1(r_g =0.2973, *P* value = 0.0439), Interleukin-17D (r_g =0.389, *P* value = 0.0381), Neurensin-1(r_g =0.3667, *P* value = 0.0333), Trypsin-2(r_g =0.3653, *P* value = 0.038), Serpin A9(r_g =0.3746, *P* value = 0.0424).

Table 1
Genetic correlations between between spondyloarthritis and human plasma proteins

spondyloarthritis	human plasma proteins	Genetic Correlation	p values
	Testis-specific chromodomain protein Y 1	0.2973	0.0439
	Interleukin-17D	0.3890	0.0381
	Neurensin-1	0.3667	0.0333
	Trypsin-2	0.3653	0.038
	Serpin A9	0.3746	0.0424

Note:The GWAS summary data for spondyloarthritis(SpA) acquired from an European cohort study, including 3966 SpA cases and 448,298 controls. GWAS summary data of human plasma proteins include a total of 3,622 human plasma proteins were quantified in 3,301 healthy participants from 25 centers across England. LD score regression software (https://github.com/bulik/ldsc) was used here to evaluate the genetic correlation between SpA and each of the human plasma proteins.

GWAS:genome-wide association study;SPA:spondyloarthritis

Screening of DEGs associated with SpA

The data set GSE57376 contains 25 samples, including 15 patients with psoriasis who did not have psoriatic arthritis and 10 patients with psoriatic arthritis. After screening the DEGs, the dataset of GSE57376 screened a total of 23982 genes with $P < 0.05$ and $|log2FC| > 1$(Fig. 1). Through compared with the 5 identified human plasma proteins, we found Interleukin-17D(IL-17D) was the DEG associated with SpA at the gene expression level.

Discussion

In recent years, some studies have proved that human plasma proteins play essential roles in the pathogenesis of SpA[8]. However, the genetic mechanism underlying the correlation remained unknown. In this study, we conducted the LD score regression analysis to explore the genetic correlation of human plasma proteins and SpA. Several proteins were found to be associated with SpA. As far as we have known, this is the first study systematically exploring the impact of human plasma proteins on SpA.

IL-17D showed the suggestive genetic correlation evidence with SpA through using the LD score regression analysis and GEO2R application. IL-17 is a novel family of inflammatory cytokines. Excessive activation of IL-17 signaling can contribute to autoimmunity or chronic inflammatory disease[19]. Some studies have shown that the IL-17 family plays an essential role in the development of SpA[20, 21]. For

example, Taams LS et al. indicated that the IL-17A inhibitors show efficacy in treating multiple facets of SpA, including psoriasis, enthesitis, synovitis, bone erosion, and new bone formation, which illustrates the importance of IL-17A in pathophysiology of SpA[21]. IL-17A is the most studied among the IL-17 family. IL-17A can degrade the extracellular matrix within the joint by inducing the production of matrix metalloproteinases (MMPs), and IL-17A can lead to active osteoclasts and destruct the bone by up-regulating receptor activator of nuclear factor-κB (NF-κB) expression[21]. What's more, IL-17A promotes angiogenesis, increasing blood flow and facilitating the influx of inflammatory cells into the joint, leading to joint inflammation[21]. Although few studies on the pathologic mechanism effect of IL-17D on SpA, both IL-17A and IL-17D belong to the interleukin-17 family. They have homology with each other, including similar amino acid sequences and structure-function. So, IL-17D may have potential mechanisms in the pathogenesis of SpA.

Trypsin-2 showed the genetic correlation evidence with SpA. Trypsin-2 can degrade the major structural collagen of articular cartilage, type II collagen, and activates several collagenases [22]. These mechanisms are capable of contributing to tissue-degenerative diseases such as rheumatoid arthritis [22]. Moilanen M et al. found trypsinogen-2 can degrade type I collagen, involving the initial action of collagenolytic matrix metalloproteinases (MMP-1, -8, and − 13) activated by MMP-3[23]. It has been reported that MMPs, and MMP-3 in particular, are produced in response to cytokines in the joints, being more highly expressed in the synovial tissues of SpA patients than in peripheral blood mononuclear cells [24]. In addition, S Sun et al. detected a significantly increased expression of alpha 1-anti-trypsin (ATA1) in synovial membranes of patients with ankylosing spondylitis [25]. Trypsin-2 may become a suggestive human plasma protein for the pathogenesis of SpA, though the precise genetic relationship requires further research.

Serpin A9 also showed the genetic correlation with SpA. Serpin A9 is a protease inhibitor that inhibits trypsin and trypsin-like serine proteases. The genetic correlations between the trypsin and SpA have already been discussed in our study above. STRING (http://string-db.org) is a database that aims to provide a critical assessment and integration of protein-protein interactions, including direct (physical) as well as indirect (functional) associations [26]. By using the STRING database, we found there were functional partnerships and interactions among Serpin A9 and germinal center-associated signaling and motility protein(GCSAM), interferon regulatory factor-4 (IRF-4). Serpin A9 can affect the GCSAM, which mediates the migration-inhibitory effects of IL-6[27]. Da-He Li et al. proved that IL-6 can up-regulate Annexin A2, which may promote ligament ossification, and down-regulating Annexin A2 can ameliorate ossification of fibroblasts from patients with ankylosing spondylitis[28]. We found the Serpin A9 could regulate the expression of interferon regulatory factor-4 (IRF-4) by using STRING database. Chen Q et al. found IRF-4-binding protein can inhibit IL-17 through controlling the activity of the IRF-4 transcription factor[29]. IL-17, known as a family of inflammatory cytokines, has already been discussed in our study above too. Although no researchers have studied whether Serpin A9 has a direct effect on SpA, our results provide a potential insight to study the genetic mechanism of SpA.

Neurensin-1 showed the genetic correlation evidence with SpA. Researchers found Neurensin-1 has an impact on the expression of growth arrest and DNA damage-inducible protein beta (GADD45B)[30]. GADD45B can encode a ubiquitously expressed protein that is often associated with growth arrest and apoptosis through demethylating CpG islands of representative gene targets[30]. Some studies have demonstrated that DNA demethylation and hypermethylation play significant roles in arthritis[31]. For example, Dániel M Tóth et l. found the DNA demethylation induced by 5'-azaC halted arthritis progression in mice, which can attribute to the inhibited production of IgG1 antibodies[31]. This study proved that DNA hypermethylation plays a leading role in the pathogenesis of autoimmune arthritis and DNA demethylation has a therapeutic potential in arthritis management. What's more, Keremu et al. found Neurensin-2 could promote osteosarcoma cell proliferation and growth by dysregulating Wnt/β-catenin signaling[32]. It was reported that the Wnt signaling is likely to play essential roles in the process of ankylosis in SpA, and the activation of β-catenin signaling in cartilage tissue may be the key event leading to spine and joint destruction in patients with SpA[33]. To sum up, the roles of Neurensin-1 in SpA are potential, and the precise relationship between Neurensin-1 and SpA requires further research.

The strength of our study is that we used the latest large-scale GWAS summary dataset of SpA to observe multiple genetic correlations between SpA and human plasma proteins through LD score regression analysis[5, 15]. The large sample size of GWAS summary data can ensure the accuracy of our research results. We verified whether human plasma proteins genetically related to SpA were differentially expressed at the gene expression level. These results were better provided the latest clues for future research on the genetic mechanism of SpA.

This study also has some limitations. Firstly, the GWAS summary data came from the UK Biobank cohort and based on European ancestry, so our results may not apply to other ancestry studies. Further LD score regression of different populations is needed to prove our results. Secondly, to validate the TWAS results, we compared the significant genes identified by TWAS of SpA with the differentially expressed genes detected by the mRNA expression profiles of psoriatic arthritis. However psoriatic arthritis is just one disease within SpA. So our results should be interpreted with caution. Further biological studies should be conducted to confirm our findings.

Conclusion

In this study, we investigated the genetic correlations between SpA and human plasma proteins via LD score regression analysis. 5 kinds of human plasma proteins that may have genetic correlations with SpA were found. IL-17D is the common gene identified for SpA both at protein level and gene expression level. Our study potentially provides a new way to study the genetic mechanism, diagnosis, and treatment of SpA in the future.

Abbreviations

AS

Ankylosing spondylitis
ATA1
Alpha 1-anti-trypsin
GADD45B
Growth arrest and DNA damage-inducible protein beta
GCSAM
Germinal center-associated signaling and motility protein
GEO
Gene Expression Omnibus
GWAS
Genome-Wide Association Study;
HLA-B27
Human leukocyte antigen-B27
IL-17D
Interleukin-17D
IRF-4
Interferon regulatory factor-4
LD score regression
Linkage disequilibrium score regression;
MMP
Matrix metalloproteinase
PsA
Psoriatic arthritis
ReA
Reactive arthritis
SNP
Single nucleotide polymorphisms;
SpA
Spondyloarthritis

Declarations

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Not applicable

Conflicts of interest

The authors declare that they have no conflicts of interest.

Authors' contributions

Author1, author5 had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Author1, Author2 designed this trial and wrote this manuscript. Author1, Author2, Author3, were responsible for the collection of data. The analysis and interpretation of all data were finished by Author1, Author4. All authors read and approved the final manuscript.

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Figures

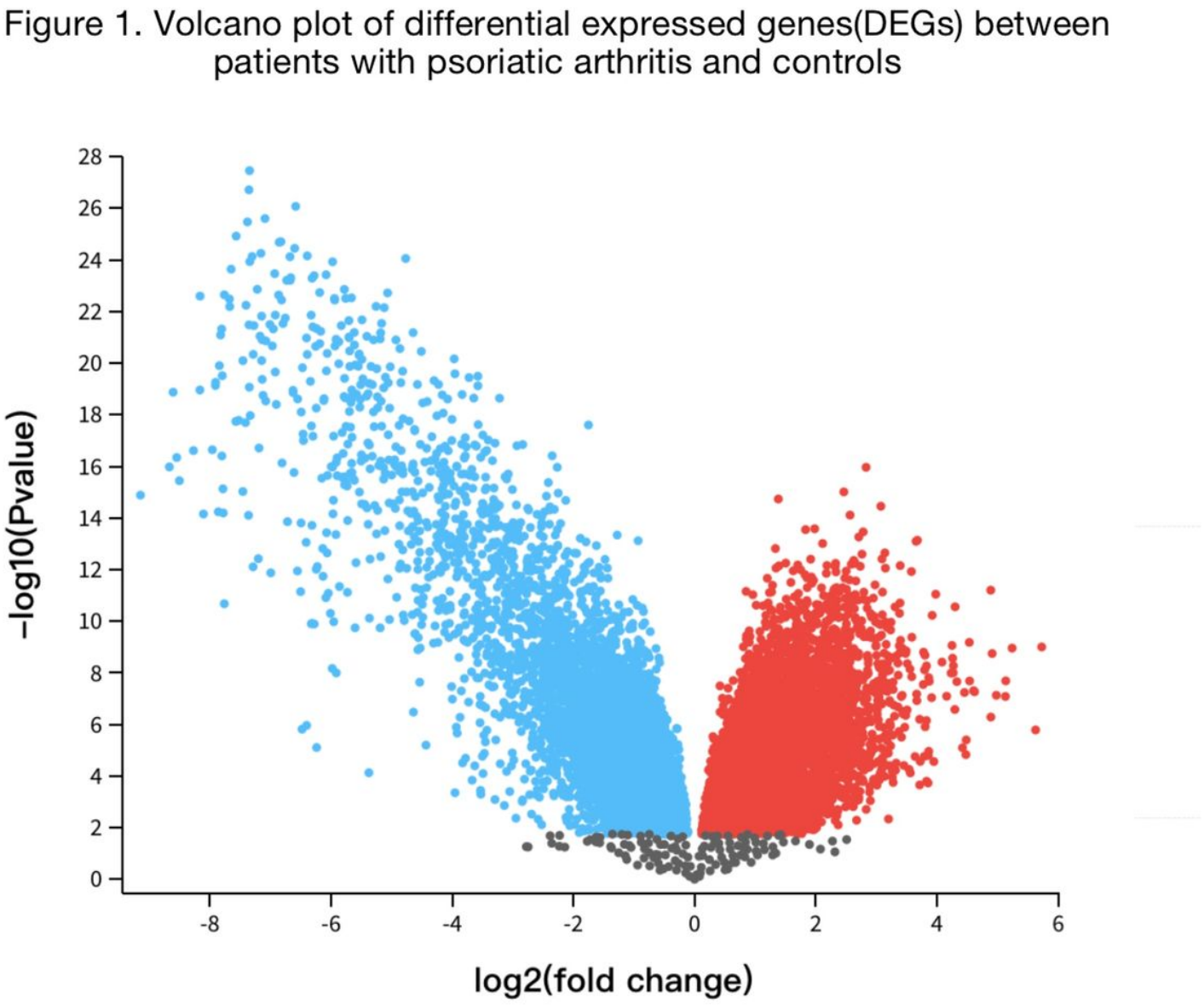

Figure 1

Volcano plot of differentially expressed genes(DEGs) between patients with psoriatic arthritis and controls

Note: The expression profile dataset of GSE57376 contains 25 samples, including 15 patients with psoriasis who did not have psoriatic arthritis and 10 patients with psoriatic arthritis. We used GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) to analysis the differentially expressed gene (DEGs). The results were output to the volcano map, in which blue represents down-regulated expression and red represents up-regulated expression.

DEG: differentially expressed gene